Relation between the health of laying hens and eggs quality: a mini review

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SUMMARY

The egg has a double functionality because of its excellent composition of nutrients and bioactive compounds with important role in preventing the chronic and infectious diseases, plus the fact that it allows the development of a new organism. The egg is a complete and versatile food. Egg consumption (100 g) covers 5-20% of the daily vitamin and mineral requirement for the man and has a good energy to protein ratio. The quality requirements include organoleptic characteristics, nutritional properties, technological and safety aspects. Within the context of and increased demand for egg consumption, and of the requirements of the quality standards, the focus is on the research of the dietary improvement of the quality, while not overseeing the aspects related to hygiene, health state and contamination. For instance, egg colour is provided through natural plant pigments, carotenoids, which also have the role of antioxidants and/or precursors for vitamin A, and xanthophylls. Yolk quality is associated to yolk colour and with the cholesterol level. Selenium-methionine supplements to layer diets increase the α-tocopherol content of the eggs, while decreasing slightly the yolk cholesterol. A way to increase rapidly the n-3 polyunsaturated fatty acids level of the hen eggs is to use linseed, canola, hempseed, camelina etc., or to supplement the diets with fishmeal or fish oil. The low dietary Ca levels affect eggshell thickness, breaking strength and form. The incidence of broken eggs ranges between 6 and 8%. The acid-base balance, influenced by the Na, K, Cl ratio in layers, can affect the breaking strength of the eggshell. The laying hens are more susceptible to diseases, and the use of antibiotics in sub-therapeutic doses to stimulate growth and/or in prophylactic doses to prevent diseases, or in therapeutic doses to cure diseases, generated problems related to the microbe resistance. Romania has some of the highest levels of antimicrobial resistance in the EU. Health systems need to respond more efficiently to changing health care needs. Giving antibiotics to sick animals will kill many

bacteria, but resistant bacteria can survive and multiply, thereby contaminating the food chain. Not without significance is the fact that the livestock health care and nutrition are the main factor affecting the quality of animal origin products, animal health, metabolic disorders incidence and animal performance. This is the reason why the research is complex, and the trend is to highlight the multiple quality and health aspects closely related to quantity and to consumer orientation.

Keywords: antimicrobial resistance, eggs, health, hens, quality

CONTEXT

Globally, poultry production has one of the highest rate of growth among the animal production industries. A bird can produce up to 320 eggs per year and uses with the highest efficiency the vegetal ingredients. The production and consumption of eggs increased slightly within the EU (>6500 mt/an (https://ec.europe.eu/agriculture). However, the sector must have a sustainable development, the production must increase at low costs, the environmental impact must be monitored and the quality must be according to consumer demands. The consumption of eggs (100 g) covers 5-20% of the daily requirement of vitamins and minerals, providing a good balance of the energy and protein. The demand for eggs is foreseen to increase by 65% (Alexandratos & Bruinsma, 2012).

Lately, the hen egg quality standards on freshness, safety, colour, composition, etc., increased. Fernandez (2016) highlighted egg functionality because of its bioactive compounds that play an important role in the prevention of chronic and infectious diseases. Actually, the hen egg has a double functionality because it allows the development of a new organism, while being a complete and versatile food. The egg is a perfectly balanced source of protein of high biological value, and a source of digestible lipids with a high content of polyunsaturated fatty acids. However, the proportion of the different fatty acids is variable (Nys and Sauveur, 2004; Seuss-Baum et al., 2011; Miranda et al., 2015). We can say that the egg has all the nutrients (Nys, 2010). Egg quality can be estimated in relation with the bacterial contamination (Englmaierová et al., 2014). The contamination of the egg surface with a large number of microorganisms, dominated by Gram-positive bacteria (4·82 ± 0·51 log CFU/eggshell in on-floor systems compared to cage system mean 4.57 ± 0·58 log CFU/eggshell, Salaün et al., 2010), raised questions on the safety of the production systems (Englmaierová et al., 2014). Thus, some farmers shifted from the classical system to the certified organic system, which no longer uses antibiotics. However, such as Cherian, (2013) showed, this raises several problems since the layers are susceptible to diseases, due to

the intense egg formation metabolism and a low immune system. The use of antibiotics to control infectious pathologies had led to high successes due to the efficiency of feeding as effect of the improved health state (Mehdi et al., 2018). Antibiotics can be used in various ways in animal production: sub-therapeutic doses to stimulate growth, prophylactic doses to prevent diseases, and therapeutic doses as cure for diseases (Lander et al., 2012). The medical evidences suggest, however, that the exaggerated use of antibiotics generated problems due to the antimicrobial resistance (AMR) (Mehdi et al., 2018). The European Antibiotic Awareness Day on 7 November (WHO, 2018) confirmed that across the EU the number of patients infected by resistant bacteria increases. Antimicrobials, such as antibiotics, are used to kill microorganisms or to stop them from multiplying. The volume of antimicrobials used in animals reared for food exceeds the use in humans worldwide, and nearly all the classes of antimicrobials that are used for humans are also being used in animals reared for food. AMR refers to the ability of microorganisms to withstand antimicrobial treatments. The State of Health Report in the EU (2017) shows that Romania has some of the highest levels of antimicrobial resistance in the EU. Not without significance is the fact that the livestock health care and nutrition are the main factor affecting the quality of animal origin products, animal health, metabolic disorders incidence and animal performance. It is expected that the transfer of knowledge on animal health and AMR to practice improve the viability of animal farms thanks to lowered production cost that may be achieved by preventive biosecurity programs without using antibiotics.

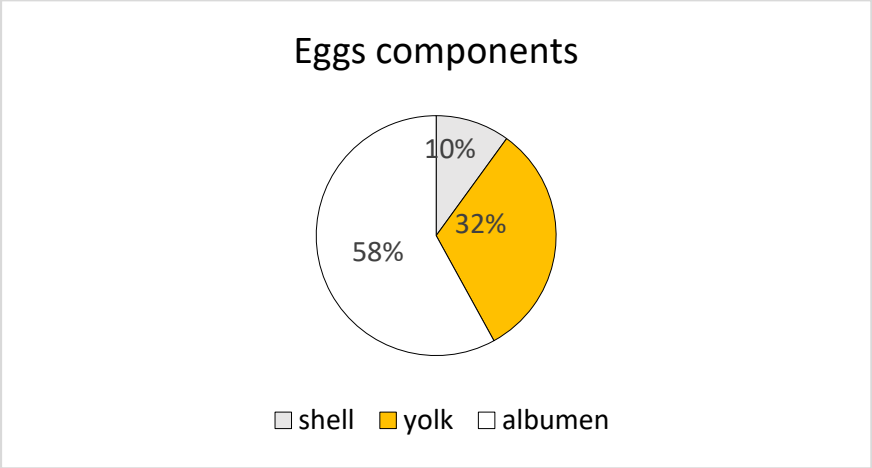
The objective of our study is to highlight aspects related to hen egg quality, to the safety and antimicrobial resistance in layers.

COMPONENCE AND QUALITY OF THE HEN EGG

A detailed analysis of the hen egg quality presumes considering many aspects due to the multitude of the factors of influence, and of the high costs required to meet the quality standards. Chukwuka (2011) defined quality as "*a property of a food that influences consumer decision to accept or reject that food*". This presumes developing in-depth research for the continuous insurance of quality, while not ignoring layer health.

The dietary nutrients are transformed by the hen, through organs and systems, into substances that become part of the eggs. The quality criteria include organoleptic characteristics, nutritional properties, technological aspects and safety aspects. Figure 1 shows the egg components (adapted after Leeson, 2006, Herron & Fernandez, 2004). The egg has no fibre, but it

has 11% proteins, predominantly in the albumen, and 11.2% lipids, predominantly in the yolk (Faris et al., 2011)



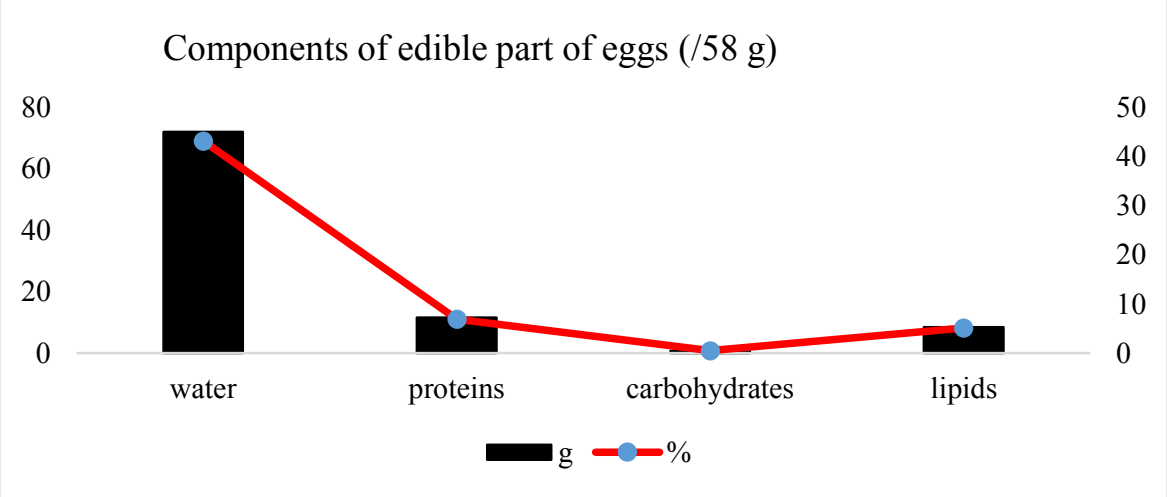


Fig 1. Eggs component; According to reported data (Herron & Fernandez, 2004)

Egg quality includes functional, aesthetic and microbiologic properties of the albumen and yolk (Coutts and Graham, 2007).

FUNCTIONAL PROPERTIES

Previous researches showed the possibility of modifying the nutritional qualities of the egg through the diet (Wang et al., 2017). This concerned amending the defects of aspect, shape, taste and smell (Wang et al., 2017), and of composition, by increasing the level of particular active biocompounds (lutein and zeaxanthin, Skřivan et al., 2015, or decreasing the cholesterol level, Salma et al., 2007). Hen egg composition in minerals and vitamins can be improved through feeding. Ca and P are the main components of the eggshell and play a major role in eggshell quality. Zn, Cu and Mn also improve eggshell quality. In average, a bird must eat about 4 g

de Ca /day, the egg containing 2 g Ca. More than half of the amount of Ca is required for the formation of the eggshell. The eggshell contains over 20 mg P, and the egg, over 120 mg P, distributed unequally between the various layers. The egg is an important exogenous source of de folate, riboflavin, selenium, choline, vitamin B12 and vitamin A, and of vitamins K and D (Naviglio et al., 2012). The dietary riboflavin, for instance, can be transferred easier than vitamin A into the egg, due to its intermediary storage into the liver (King'Ori, 2012). The addition of fat improves the absorption of the fat-soluble vitamins (El-Huseiny, 2008). Selenium plays and active biological role in poultry feeding, and its availability is affected by methionine, thiols, heavy metals, and vitamin C (Suchý et al., 2014). In the feed, it can be either in organic and/or inorganic form. The selenium-methionine (Se-Met) supplementation of the poultry diets increases the α-tocopherol level in the egg and decreases slightly the cholesterol level of the yolk (Baylan et al., 2010, Canoğullari et al., 2010, Suchý et al., 2014). The eggs enriched in organic Se provide a functional character to the eggs, by the super-standard levels of Se.

The consumption of eggs is controversial in the literature. If we take into consideration that the highest amount of cholesterol intake per day must be 100 mg / 1000 Kcal energy diet, it results that one can eat 2 -3 eggs per week (Spence et al., 2010). DiMarco et al., (2017) determined that 1 egg/day improved HDL composition and function, and that at 2–3 eggs/day the functions and composition of these lipoproteins are associated with the increased concentration of lutein and zeaxanthin. The egg yolk contains about 275 mg of cholesterol (Spence et al., 2010), and for the entire egg USDA, (2000) reported a concentration of 213 mg cholesterol. On the other hand, the studies of Aljohi et al., (2019), suggested that a consumption of 12 eggs per week for 1 year did not influence the serum concentration of lipids, apolipoprotein, lipoprotein and high sensitive C-Reactive Protein in people with macular degeneration but who were healthy otherwise. It is certain that the consumers became aware of the adverse effects of the saturated fatty acids and cholesterol, which can increase the risk of cardiovascular diseases although, if we compare it with other protein sources, proportionally it has less saturated fats (Naviglio et al., 2012). The scientists and doctors make, however, distinction between the dietary and circulating cholesterol, and their non-linear relationship (Aljohi et al., 2019). The literature shows that the sources of dietary fat influence the lipid composition of the cell membrane, tissues and eggs yolk (Laudadio et al., 2015), as well as the egg or yolk cholesterol (Millet et al. 2006). For instance, Salma et al., (2007) used Rhodobacter capsulatus (bacterial cell grown in outdoor culture under natural illumination) to decrease the egg cholesterol as the HDL-cholesterol level increases. The marigold flower (Tagetes erecta L.) extract was included into the diet by

Skřivan et al., (2015) to improve the content of lutein and zeaxanthin. Furthermore, researches aimed to identify plant resources that can improve egg composition in polyunsaturated fatty acids (PUFA), including a-linolenic (ALA, C18:3 n-3), eicosapentaenoic (EPA, C20:5 n-3), docospentaenoic (DPA, C22:5 n-3) and docosahexaenoic (DHA, C22:6 n-3) acids (Antruejo et al., 2011, Panaite et al., 2016). A way of increasing the egg content of n-3 is to use linseed, canola, hempseed, camelina etc., or fishmeal and fish oil (Yannakopoulos, 2007, Fraeye et al., 2012, Wang et al., 2017). At a dietary level of 3-4% fish oil, the DHA level of the egg reached 180-250 mg (Wang et al., 2017). The dietary 5% flaxseed meal and 2% camelina meal, with 3 different levels of Cu supplements (75 mg /Kg, 100 mg/Kg, 150 mg/Kg, Panaite et al., 2016) increased significantly DHA level in the egg yolk. Yolk DHA concentration increases, than it reaches a plateau level following the use of hemp seeds and hemp seeds oil (Gakhar et al., 2012). The antioxidant capacity is correlated positively with the content of n-3 PUFA. The biologic antioxidants react with the free radicals or with other precursor metabolites converting them into less reactive molecules (Phaniendra et al., 2015).

AESTHETIC PROPERTIES

The outer look of the egg is important for the consumer and it presumes taking into consideration the following characteristics: thickness (300-350 micrometer thick), cleanliness, shape, texture, structure and soundness. An outer look that does not fit the quality standards leads to great losses. EU Council (2006) set two quality classes for the eggs, A and B, while the USA Department of Agriculture developed 3 classes. Generally, class A eggs can be given directly into consumption, while class B eggs must be processed (Chukwuka et al., 2011).

The eggshell is porous and it has a perfectly ordered structure with polycrystalline organization throughout the calcified shell. The eggshell contains 3.5% organic matter, consisting of the membrane and particular constituents of the $CaCO_3$ (95%) layer, as calcite (Nys & Gautron, 2007). During the formation of the egg, a mass of inorganic minerals is deposited (Kheirkhah et al., 2017). The inner eggshell membrane (~20 μm) separates the eggshell from the albumen. The other membranes, with a thickness of about 50 μm, are located between the inner membrane and the calcified part of the eggshell. The membranes are made of organic fibres (Nys & Gautron, 2007). Calcium is of the highest importance for the eggshell quality. A low level of dietary Ca affects eggshell thickness, breaking strength, and shape. The incidence of broken eggs ranges between 6 and 8%. The acid-alkali balance influenced in layers by the Na, K and Cl ratio,

can affect the eggshell breaking strength. Egg colour cannot modify its nutritive value or taste.

MICROBIOLOGICAL PROPERTIES AND MICROBIAL CONTAMINATION

There are multiple routes of microbial contamination of the eggs. Contamination can occur before laying or after laying, upon contact with the exterior. The first route of contamination are the eggshell pores. Eggshell is the best barrier against bacterial contamination, but the contamination possibility still exists if they are not used properly, or if the refrigeration or processing conditions are improper, or if the growing conditions do not observe the technological conditions. The egg has several components (cuticle, shell, shell membranes, a pH of the albumen which is typically after laying 7.6-7.9, lysozyme and conalbunen, which have bactericide properties), which decreases its perishability compared to other foods (Abeyrathne et al., 2013, Rahman, 2013). The cell membrane provides the best protection against bacterial penetration from the outer environment into the egg, but once inside, they replicate at a low rate because of the albumen protein viscosity, the pH and the bactericide properties of the lysozyme and conalbumen (Guyot et al., 2016). De Reu et al. (2006) stated a limit of 5 log CFU/egg for an egg to be accepted in terms of hygienic quality. Many pathogen microorganisms have been identified on the eggshell and in the egg: *Listeria monocytogenes, Yersinia enterocolitica, Escherichia coli, Salmonella and Campylobacter* spp. (Salihu, Garba & Isah, 2015). The intestinal tract is the main reservoir of *Salmonella*. Fungi from the classes of *Aspergillus*, *Penicillium* etc., aflatoxins can also be pathogens for egg contamination. *E. coli* is extensively used to monitor AMR in food animals (Nhung et al., 2017). Given the rate of lead dispersion, it can contaminate the eggs, the yolk particularly, which is a potential human health hazard, as highlighted by Trampel et al., (2003). The antibiotics, antibacterial enzymes, the iron-sequestering and bacterial protease-inhibiting proteins, are ways of controlling pathogenicity before laying. The dietary curcumin increases the level of antioxidants from the eggs, which reduces lipid peroxidation and helps coccidiosis control (Galli et al., 2018).

ANTIMICROBIAL RESISTANCE IN LAYING HENS & ALTERNATIVES TO ANTIBIOTICS

Given the large number of types of animal foods identified as reservoirs of bacteria that produce infections in humans, a major challenge for the current researches is the development of viable alternative solutions to antibiotics. The natural bacterial resistance appears when an antibiotic cannot attack particular receptors of the bacterial cell if those bacteria do not have adequate receptors.

Part of the problems caused by the antimicrobial resistance in the EU are due to the antibiotics used in animal feeding. The antibiotics used as treatment or for the prevention of infections in animals are from the same chemical group with those used for humans. The need to control AMR joined the efforts upon WHO suggestion, and in consequence, in 2018, the National Committee for Limiting Antimicrobial Resistance was established in Romania. The purpose of this Committee is to develop and monitor the implementation of the National Strategy on the prudent use of antibiotics, to limit the increase of AMR in the population and animals and to prevent the spread of resistant microorganisms at national level. Health systems need to respond more efficiently to changing health care needs driven by demographic changes and exploit more fully the potential of new digital technologies to strengthen prevention and care. Giving antibiotics to sick animals will kill many bacteria, but resistant bacteria can survive and multiply, thereby contaminating the food chain. In Romania, 52% of the people are total agree that sick farm animals to be treated with antibiotics if this is the most appropriate treatment, 35% are total disagree while 13% don't know. At the same time Mehdi et al., (2018) showed that antibiotics cure infections caused by bacteria, but they are not efficient against fungi and viral pathogens.

The main contaminants are Gram-negative bacteria from the *Enterobactericiae* family, although *Staphylococcus, Streptococcus* and *Bacillus* spp. were isolated in in a large proportion in addition to *Aspergillus spp* (Salihu, Garba & Isah, 2015). Some bacteria, such as *Salmonella* and *Campylobacter*, are associated to the intake of contaminated food coming from antibiotic-resistant animals. *Campylobacter* is a major cause for food-borne diarrheal diseases in humans, and in the old people and children, the infection caused by these bacteria can be fatal Mehdi et al., (2018). *Escherichia coli* can be found both on the eggshell surface and inside the egg. *Escherichia coli* is one of the bacteria that cause serious urinary infections, pneumonia, meningitis and peritonitis in humans (Salihu, Garba & Isah, 2015).

Through the contaminated eggs, these bacteria are often transmitted to humans, where they proliferate in the intestine. Although on the outside the eggs seem normal, properly hygienically, yet they may contain germs. Habrun et al., (2012), developed a set of prevention and control measures: hygienic and biosafety measures, decontamination of water and feed, use of fatty acids and additives in poultry feed and use of vaccines, prebiotics and probiotics. Probiotics can destroy the pathogen microorganisms due to the antimicrobial compounds they produce, such as bacteriocins and organic acids; they can contribute to the dominance of the beneficial microorganisms to the detriment of the pathogen ones within the gastrointestinal tract, they stimulate the immune response, improve

nutrient digestion and absorption. The most used bacterial strains for probiotics are *Bacillus, Lactobacillus, Lactococcus, Streptococcus, Enterococcus, Pediococcus, Bifidobacterium, Bacteroides, Pseudomonas, yeast, Aspergillus,* and *Trichoderma*, etc. (Ayaşan, 2013, Ayukekbong et al., 2017) .

The active biocompounds from plants and extracts with high content of nutricines are solutions to replace the antibiotics, particularly for the ecological layer farms. The essential oils of thyme (*Thymus vulgaris*), or carvacrol (component of oregano, *Origanum vulgare*), garlic, respectively have potential to control the diseases produced by bacteria (Griggs and Jacob, 2005). The organic acids (formic, acetic and propionic acids) added to the drinking water or to the feeds for poultry are efficient solutions to replace antibiotics, showing good effects against colonization by *Salmonella* or *Escherichia coli*.

FEED EFFECTS ON HENS EGG QUALITY

Many studies investigate the additional health benefits of the nutrients (Yenice et al., 2016, Alagawany et al., 2016). The maintenance of the immune functions depends on the nutritive principles. Several nutritional characteristics of the egg were the target of modulation through layer feeds, given the connection between the feed, digestive functions, quality and production. A correct nutrition is the key to a state of physical and physiological comfort, which leads to optimal performances, proper health state and good welfare. An adequate nutritive balance will avoid the excessive mobilization of body reserves, will supply nutrients at an optimal level, remedies the physiological imbalances, reduces the onset of infectious disorders by consolidating the integrity of the tissue cells and will optimise the immune defence mechanisms. The feed intake, with its multifunctional complexity and economic importance, plays a basic role as variable of interpreting the nutritional response. Energy is an important nutritional component, which derives from the oxidation of carbohydrates, fat and proteins (The Merk Veterinary Manual, 2010). The nutrients which ensure the requirement for maintenance and production are key criteria which the nutritionists use to optimise the quality and production performance. Of the nutritive principles, protein has a primordial role for building and repairing muscles, organs, skin, feather and other body tissues; needed to produce hormones, enzymes and antibodies; the protein in eggs is easily absorbed by the body. A proper level of dietary protein is necessary to ensure the different functions of the organism, such as the maintenance of the reference markers for immunity within normal limits and ensure protection against diseases Alagawany et al., (2016).

The hen egg contains all 9 essential amino acids. The notion of perfectly balanced protein is often expressed as ideal protein – a mixture of proteins and amino acids which meets exactly the requirement of the birds for each amino acid (essential or non-essential), which means no deficiency or excess (Wijtten et al., 2004). In practice this is possible only by using proteins with high biological value supplemented with synthetic amino acids. As shown by Alagawany et al. (2016), the level of dietary protein for layers does not affect significantly the composition of the egg albumen (moisture content albumen solids, dry albumen, albumen protein and nitrogen free extract), except for the organic matter and ash ($P< 0.01$).

The deficient dietary minerals and vitamins can cause health problems, which is why they have to be supplied at the proper level. Thus, the dietary calcium level (3 g in the eggshell) and P level are highly important for eggshell formation and for bone mineralization. The introduction of vitamin D into the feeds is essential for Ca metabolism. The deficiency of vitamins C, E and A can lead to adverse effects (Ahmadi and Rahimi, 2011). A high dietary level of non-starch polysaccharides increases the intestinal viscosity, being necessary to add enzymes to the diet, which also act on egg quality. Yolk quality is associated to the colour and cholesterol content. Egg yolk contains about 16% protein, 35% lipids and 0.2% lipophilic pigments (Kopřiva et al., 2014). The colour, for which Vuilleumier, (1969) developed the Roche Yolk Colour Fan (RYC on a range of 1-15), is provided by natural pigments from plants – the carotenoids (which also have the role of antioxidants and/or precursors of vitamin A) and xanthophylls. The pigment is transferred directly from the feed to the egg. Corn is an excellent source of natural xanthophylls, giving the yellow colour of the yolk, while the cereal grains with no pigments (sorghum, wheat and barley) give a pale yellow colour of the yolk. The most important xanthophylls for yolk colour are zeaxanthin and lutein. The *Chlorella* biomass supplements to layer diets increased the carotenoids level in the albumen (lutein and zeaxanthin accounted for 90% of the pigments, Kotrbáček et al., 2013). Lokaewmane et al., (2011), improved yolk colour (11.69 RYC) using an extract of paprika. Chow et al., (2014), validated the hypothesis that a particular score of yolk colour can be achieved when the carotenoid molecules are emulsified by microemulsion nanotechnology. In the same year, because some countries do grow corn (Iran, for instance), Shahsavari (2015) proposed diets based on wheat and barley supplemented with 5% alfalfa meal for an acceptable yolk colour, but with poorer performance. The 2% dietary red pepper can also lead to good yolk pigmentation (14.3 RYC).

Beyond these aspects, the egg yolk has a bad reputation due to the cholesterol level, but the opinions are controversial. Furthermore, Fernandez, (2010) suggested that the effects of eating an egg per day are not adverse in terms of the blood cholesterol, and Andersen et al., (2013)

documented an increase of the plasma HDL–cholesterol. Several studies (Spence et al., 2010, Naviglio et al., 2012) showed that a consumption of 1-2 eggs per day, on the background of a low dietary fat level, has no adverse influence on the lipid profile, maybe also because of the low level of saturated fats in the egg. However, one must not oversee the functions of the cholesterol in the organism (such as the production of bile acids necessary for the digestion of fats and which help the absorption of the fat-soluble vitamins A, D, E and K (Naviglio et al., 2012).

A study was conducted by Olteanu et al., (2016) in order to establish antioxidant efficiency of dietary grape seed meal associate with n-3 PUFA and vitamin E. A strong correlation between antioxidant capacity and the concentrations of polyphenols (R2 = 0.9725) and of flavonoids (R2 = 0.9948) was found. The ω-6/ω-3 PUFA ratio decreased while polyphenol concentration of methanolic yolk extracts increased.

Manda et al., (2008) conducted a clinical study on 62 people who consumed 6, n-3 PUFA enriched eggs/week, for 6 weeks. The eggs were enriched through dietary flaxseeds given to layers. The conclusion of the study was that the eggs enriched in PUFA, with 2.65 g/100 g acid linoleic (ALA) (about 5 times more than the conventional eggs), had beneficial effects on consumer health state by the significant lower level of blood plasma triglycerides and fibrinogen.

Conclusions

Of the foods of animal origin, the egg stands out due to its two-fold functionality given by the composition in nutrients and active biocompounds, on the one hand, and by the fact that it allows the formation of a new organism. The egg has almost all the nutrients, the protein has a high biological value, the minerals and vitamins are an important part of its composition, while the structure of lipids is variable. The egg quality researches are of great interest and continue to expand because of the increasing demand for consumption eggs, associated to increasingly higher quality standards. Without ignoring quantity, the studies focus on the functional, organoleptic and aesthetic properties, on the microbial contamination associated to antibacterial resistance. All these converge towards the possibility of interventions through food, towards observing the hygiene rules, towards eggshell quality to reduce the breaking percentage, towards the internal quality by improving the content in particular nutrients from the feed ingredients, towards a good health state of the stock of birds, necessary to avoid prejudices to the poultry production industry.

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